AN EVALUATION OF THE ENZYTEC® PESTICIDE DETECTOR TICKET FOR USEFULNESS IN FIELD MONITORING OF PESTICIDES AND IN THE REGULATORY PROGRAMS OF THE CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE

by

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ENVIRONMENTAL HAZARDS ASSESSMENT PROGRAM

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EXECUTIVE SUMMARY

Midwest Research Institute (MRI) has recently introduced a new product, the EnzyTec® Pesticide Detector "ticket." The California Department of Food and Agriculture (CDFA) evaluated the EnzyTec® Pesticide Detector for its usefulness in the Department's regulatory programs and for its overall usefulness in detection of pesticide residues in the field. This report is the result of this evaluation.

The tickets test for the presence of cholinesterase-inhibiting chemicals. If such a chemical is present at levels above the Minimum Detectable Level (MDL) of the ticket, the ticket changes color. The tickets do not indicate which cholinesterase-inhibiting chemical is present, nor do they indicate how much chemical is present.

CDFA evaluated the tickets for usefulness in the following departmental branches:

1. Chemistry Laboratory Services
2. Environmental Monitoring
3. Pesticide Use Enforcement
4. Worker Health and Safety

Based on these evaluations, this study concluded that the EnzyTec® as currently formulated will not meet the requirements of the CDFA for use in its regulatory programs nor can CDFA recommend their use by untrained personnel in the field.

Specific findings of this report include:

1. The tickets work well for simple water sample screening. However, as presently formulated, the MDL of the tickets for pesticides is well above the MDL needed by CDFA's regulatory programs.

2. The tickets often produce an ambiguous color change at or near their limit of detection, even with standard solutions. This ambiguity can lead to different interpretations by different users.

3. The tickets do not work well with highly colored commodities or products. MRI has developed an experimental procedure using ether or hexane that may overcome this problem. However this procedure must be done in a laboratory by trained personnel and additional experimental work is needed before it can be used routinely.

4. The tickets are not reliable for testing food commodities or products which are moderate to very acidic (pH less than about 3) or moderate to very basic (pH greater than about 8).

5. The tickets give results which may be useful for checking re-entry intervals into sprayed fields, if the user understands their limitations.

6. The tickets are not practical for field use. Many commodities must be mechanically or electrically blended before testing.

7. The tickets only detect those pesticides which inhibit cholinesterase - the carbamate and organophosphate pesticides. They will not detect pesticides such as DDT, simazine or DCPA, all of which are of regulatory concern to CDFA.

8. The tickets would not be useful to CDFA as a screening device to determine if chemical analysis of a sample is needed, since their MDL is too high and they cannot detect many kinds of pesticides.

9. Because of all the limitations (pH, pigmentation, etc.) the tickets should most likely be used only by trained personnel in situations where the user knows that a cholinesterase-inhibiting pesticide has been applied.

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We wish to thank Midwest Research Institute for providing information on the development and use of their product and for technical assistance in its use.

TABLE OF CONTENTS

I. INTRODUCTION

Midwest Research Institute (MRI) has recently introduced a new product, the EnzyTec® Pesticide Detector "ticket." This product was originally developed under contract for testing the efficiency of mobile water purification systems for the removal of specific cholinesterase inhibiting substances. The tickets utilize the inhibition of the enzyme cholinesterase coupled with a color producing reaction to detect cholinesterase-inhibiting substances. The tickets consist of two adsorbant disks, one containing substrate and the other the enzyme cholinesterase. The disks are permanently fastened to a plastic ticket which can be folded in half to bring the surfaces of the disks into contact with each other (See Appendix I).

The California Department of Food and Agriculture (CDFA) became aware of the tickets through the Department's continuing search for improved methods of pesticide analysis. After discussions with the manufacturer, the CDFA began an evaluation of the EnzyTec® Pesticide Detector for its usefulness in the Department's regulatory programs and for its overall usefulness in detection of pesticide residues in the field. This report is the result of this evaluation and is intended to apply solely to the usefulness of the EnzyTec® Pesticide Detector in CDFA's regulatory programs, and to its usefulness in field detection of pesticide residues. No other applications were evaluated nor are they implied. The EnzyTec® Pesticide Detector tickets used in this evaluation were purchased from Enzytec, Inc., 425 Volker Blvd., Kansas City, Missouri.

Outline of Report

This report will consist of a brief introduction to some of the features of the tickets followed by individual sections on the evaluations of the tickets done by the Chemistry Laboratory Services, Environmental Monitoring, Pesticide Use

Enforcement and the Worker Health and Safety Branches of the CDFA's regulatory program. The final chapter offers conclusions and recommendations.

Ticket Features

In order to evaluate the tickets properly, it is important to know any limitation or drawbacks of the tickets. The following information was provided by the manufacturer of the ticket.

1. The ticket is designed to produce a color change only when no cholinesterase-inhibiting substance is present. A color change will not occur if a cholinesterase-inhibiting substance is present or the ticket is not functioning properly. This fail-safe feature of the tickets means that the user can always be sure that negative results are accurate, although positive results may sometimes be inaccurate. Therefore, MRI recommends running tests with a minimum of two tickets to insure against a malfunctioning ticket.

2. Since they were designed to test the efficiency of water purification for the removal of cholinesterase-inhibiting substances, the tickets detect only those pesticides which inhibit cholinesterase. The two major classes of pesticides which act as cholinesterase inhibitors are organophosphates and carbamates. Although all pesticides in these two classes will inhibit the enzyme cholinesterase, the strength of this inhibition will vary from pesticide to pesticide. The minimum detection level (MDL) of the ticket for any given pesticide will depend on the pesticide's strength as a cholinesterase inhibitor. For example, a weakly-inhibiting pesticide will have to be present at a much higher concentration than a strongly-inhibiting pesticide in order to be detected by the ticket. A positive reading does not indicate how many different cholinesterase-inhibiting pesticides may be

present since the results of the ticket testing will be the sum of all cholinesterase-inhibiting pesticides that may be present in the sample.

3. The results of the tickets are semi-quantitative. This means the tickets will give a positive reading above a certain concentration of pesticide, or sum of concentrations of pesticides if more than one is present, and will give a negative reading below a certain concentration of pesticides. A positive reading will tell the user nothing about the concentration of pesticide (or pesticides) present except that it (or its sum) meets or exceeds the minimum detection limit of the ticket.

4. Since the tickets were designed for water testing, they are calibrated to measure pesticide concentration in parts per million in water solution. The tickets can be used for wiping a surface or collecting non-aqueous samples, but the results will be purely qualitative (i.e. they will show only that the pesticide is or is not present in sufficient quantity to detect, but the concentration present will be unknown).

5. The tickets may not be reliable where the pH of the sample is below about 3.0 (moderately acidic to very acidic) or above about 8.0 (moderately basic to very basic).

II. CHEMISTRY LABORATORY SERVICES STUDY

The Chemistry Laboratory Services Branch in the CDFA is responsible for all chemical analyses of samples collected by the CDFA or sent in by County Agricultural Commissioners. The Branch's main laboratory in Sacramento performed tests on the quantitative and methodological aspects of ticket analysis.

Objectives

The objectives of this study were (1) to determine how the tickets responded at or near their MDL (i.e., was the color change from no color to blue an unambiguous transition or a more gradual, ambiguous change), (2) to conduct a limited study of the effects of various pH (from pH 3 to pH 9) on the MDL of the tickets since the tickets often give false positive readings below pH 3 or above pH 8, and (3) to evaluate a new method developed by the manufacturer for using the tickets on highly pigmented commodities (i.e., produce samples) or water.

Sampling Plan

Unlike the other studies presented later in this report, this study was entirely performed in the laboratory and did not involve field collection of samples; therefore, a sampling plan is not presented. Instead, general details on the study will be presented:

1. All serial dilutions were made using distilled water. The pesticides tested were phosmet, mevinphos, ethyl parathion, carbaryl, azinphos-methyl and aldicarb.
2. Each analysis was repeated three times using a new ticket each time.
3. The pH of each solution was adjusted to the appropriate pH (3, 5, 7 or 9) using either hydrochloric acid or sodium hydroxide as needed.

Results and Discussion

The results of the serial dilution study are presented in Table I and the results of the pH study are presented in Table II.

Table I. Results of Serial Dilution Studies.

Pesticide Tested	Concentration (ppm)	Results of Ticket #1[a]	Results of Ticket #2[a]	Results of Ticket #3[a]
Phosmet	0.13	WC/BE	WC/BE	WC/BE
	0.22	WC/BE	WC/BE	WC/BE
	0.44*	W	W	WC/BE
	0.88	W	W	W
Mevinphos	0.9	WC/BE	WC/BE	WC/BE
	1.1*	WC/BE	WC/BE	W
	1.4	W	W	W
Ethyl Parathion	1.2	PB	WC/BE	PB
	1.8	WC/BE	WC/BE	PB
	2.0*	W	W	W
	2.4	W	W	W
Carbaryl	4.3	75%B	PB	75%B
	5.1	WC/BE	WC/BE	PB
	5.7	WC/BE	WC/BE	WC/BE
	8.2	W	WC/BE	WC/BE
	10.2*	WC/BE	W	WC/BE
	13.4	W	W	WC/BE
Azinphos-methyl	0.1	PB	PB/W	PB
	0.2*	W	WC/BE	WC/BE
	0.5	W	W	W
	1.3	W	W	W
	2.5	W	W	W
Aldicarb	0.1	WC/BE	WC/BE	WC/BE
	0.2	WC/BE	WC/BE	WC/BE
	0.5*	WC/BE	WC/BE	WC/BE
	1.0	W	W	W
	3.0	W	W	W

a. Tickets color read as: WC = White center
BE = Blue edge
B = Blue
W = White
PB = Pale blue

(e.g. WC/BE = white center with blue edge)
Where only one letter appears, the whole ticket was that one color.

* Defined as minimum detection level (MDL) of ticket for the pesticide tested.

Examination of Table I shows that the first objective of this study was answered. The color change is ambiguous at the MDL for the pesticides studied. The tickets gave a gradual color change, producing a mixed white and blue pattern at the MDL (see Table I).

Objective two was answered to some extent. There are no gross effects of pH on the MDL of the tickets for the pesticides studied (see Table II). However, the color change was so ambiguous that subtle effects of pH could not be detected.

MRI supplied protocols for using the tickets on highly pigmented test samples (Appendix II). These protocols involve extracting the sample with ethyl ether or hexane. Chemistry Laboratory personnel reviewed the procedures and observed that the proposed extraction procedure is similar to existing extraction procedures currently used chemical analysis. There appears to be no advantage in using the proposed procedure in the chemistry laboratory, since once an extraction has been done, an analysis by conventional instrumentation would not require much more additional time and would give qualitative and quantitative results whereas the ticket would only give qualitative results. Additionally, since hexane and ethyl ether are highly volatile and flammable, they are most safely used in controlled laboratory conditions. The method of extraction as outlined by MRI might prove impractical for field use.

MRI has suggested that the main use of the ticket by the CDFA Chemistry Laboratory might be for the screening of a large number of samples for a specific cholinesterase-inhibiting pesticide. Such screening would be followed by analysis of the positive samples found in the screening by conventional chemical analysis. As currently formulated, the MDL of the ticket is much higher than

Table II. Results of study of pH effects on the sensitivity of the tickets

Pesticide Tested	pH	Pesticide Concentration (ppm)	Results of Ticket #1[a]	Results of Ticket #2[a]	Results of Ticket #3[a]
Phosmet	3.0	0.1	WC/BE	WC/BE	WC/BE
		0.2	WC/BE	WC/BE	WC/BE
		0.4*	W	W	WC/BE
		0.9	W	W	W
		1.8	W	W	W
	5.0	0.1	WC/BE	WC/BE	WC/BE
		0.2	WC/BE	WC/BE	WC/BE
		0.4*	WC/BE	WC/BE	W
		0.9	W	W	W
	7.0	0.1	WC/BE	WC/BE	WC/BE
		0.2	WC/BE	WC/BE	WC/BE
		0.4*	WC/BE	WC/BE	WC/BE
		0.9	W	W	W
	9.0	0.1	WC/BE	PB	WC/BE
		0.2	WC/BE	WC/BE	WC/BE
		0.4*	W	WC/BE	WC/BE
		0.9	W	W	W
Carbaryl	3.0	5.1	PB	WC/BE	WC/BE
		8.2	WC/BE	WC/BE	WC/BE
		10.2*	WC/BE	WC/BE	WC/BE
		13.4	W	W	W
	5.0	5.1	WC/BE	WC/BE	WC/BE
		8.2	WC/BE	WC/BE	WC/BE
		10.2*	W	W	W
		13.4	W	W	W
	7.0	5.1	WC/BE	WC/BE	PB
		8.2	WC/BE	WC/BE	WC/BE
		10.2*	WC/BE	WC/BE	W
		13.4	W	W	W
	9.0	5.1	WC/BE	WC/BE	WC/BE
		8.2	WC/BE	WC/BE	WC/BE
		10.2*	WC/BE	W	WC/BE
		13.4	W	W	W
Azinphos-methyl	3.0	0.05	PB	PB	PB
		0.1	WC/BE	WC/BE	WC/BE
		0.2*	WC/BE	WC/BE	WC/BE
		0.5	W	W	W
	5.0	0.05	PB	WC/BE	PB
		0.1	WC/BE	WC/BE	WC/BE
		0.2*	W	W	W

Table II
Page 2

Pesticide Tested	pH	Pesticide Concentration (ppm)	Results of Ticket #1[a]	Results of Ticket #2[a]	Results of Ticket #3[a]
	7.0	0.05	PB	PB	PB
		0.1	WC/BE	WC/BE	WC/BE
		0.2*	WC/BE	WC/BE	WC/BE
		0.5	W	W	WC/BE
	9.0	0.05	PB	PB	PB
		0.1	WC/BE	PB	WC/BE
		0.2*	WC/BE	WC/BE	WC/BE
		0.5	W	WC/BE	W
Ethyl Parathion	3.0	1.9	PB	PB	PB
		2.1*	WC/BE	WC/BE	WC/BE
		2.5	WC/BE	WC/BE	WC/BE
		2.8	WC/BE	WC/BE	WC/BE
		3.1	WC/BE	WC/BE	WC/BE
	5.0	0.4	B	WC/BE	B
		0.6	PB/W	B	PB/W
		1.2	WC/BE	WC/BE	WC/BE
		1.8*	WC/BE	WC/BE	WC/BE
	7.0	1.2	PB/W	WC/BE	PB/W
		1.9*	WC/BE	WC/BE	WC/BE
		2.5	WC/BE	WC/BE	W
	9.0	0.6	PB/W	B	B
		1.2	WC/BE	WC/BE	WC/BE
		1.9*	WC/BE	WC/BE	WC/BE
Aldicarb	3.0	0.2	WC/BE	WC/BE	
		0.5*	WC/BE	WC/BE	
		0.7	W	WC/BE	
		1.0	W	W	
		1.2	W	W	
	5.0	0.2	WC/BE	WC/BE	
		0.3	WC/BE	WC/BE	
		0.5*	W	WC/BE	
		0.7	W	W	
	7.0	0.2	WC/BE	WC/BE	
		0.3	WC/BE	WC/BE	
		0.5*	W	W	
		0.7	W	W	

Table II
Page 3

Pesticide Tested	pH	Pesticide Concentration (ppm)	Results of Ticket #1[a]	Results of Ticket #2[a]	Results of Ticket #3[a]
	9.0	0.2	WC/BE	WC/BE	
		0.3	WC/BE	WC/BE	
		0.5*	W	W	
		0.7	W	W	
Mevinphos	3.0	0.9	WC/BE	WC/BE	WC/BE
		1.1*	WC/BE	W	WC/BE
		1.4	W	W	W
	5.0	0.9	WC/BE	WC/BE	WC/BE
		1.1*	W	WC/BE	W
		1.4	W	W	W
	7.0	0.9	WC/BE	WC/BE	WC/BE
		1.1*	W	W	WC/BE
		1.4	W	W	W
	9.0	0.9	WC/BE	WC/BE	WC/BE
		1.1*	WC/BE	W	WC/BE
		1.4	W	W	W

a. Tickets color read as: WC = White center
BE = Blue edge
B = Blue
W = White
PB = Pale blue

(e.g., WC/BE = white center with blue edge)
Where only one letter appears, the whole ticket was that one color.
* Defined as minimum detection level of ticket for the pesticide tested.

existing allowable pesticide levels on produce (see Table III for examples). Such screening therefore would not detect samples with higher than allowable pesticide levels and would require that all ticket results be checked by reanalysis using conventional chemical analysis. Use of the tickets as presently formulated as a screening tool by the CDFA would not save either time or money.

III. ENVIRONMENTAL MONITORING STUDY

The Environmental Hazards Assessment Program (EHAP) conducts environmental monitoring studies for the CDFA.

Objectives

The objectives of this study were: 1) to evaluate the practicality of using the tickets in the field to test well water samples and, 2) to compare the results of conventional chemical analysis with results obtained from use of the tickets.

Sampling Plan

The study used wells selected for sampling in a May 1986 survey of pesticides in well water in Tulare County. Samples from 11 wells were collected and tested for the pesticides simazine, atrazine, prometon, diuron and bromacil using conventional chemical analytical methods. Samples were also screened chemically for organophosphate, chlorinated hydrocarbon, and carbamate pesticides. Procedures used in sampling and analysis will not be presented here, but are detailed in the protocol for the pesticide survey (available from Environmental Monitoring and Pest Management Branch, CDFA).

For each of the 11 wells, six water samples were collected at each site and tested using pesticide detector tickets. The remaining water was sent to a laboratory for conventional chemical analysis. Enzyme ticket testing was

Table III. Sensitivity of tickets compared to residue tolerances for some common pesticides.

Pesticide	MDL (ppm)	Minimum Lowest Established Tolerances (ppm)	Representative Commodities
Aldicarb	1	0.002	Milk
		0.010	Meat, fat
		0.020	Soybean, sugarcane
		0.050	Grain, nuts
		0.100	Sweet potato
		0.500	Grape (pending)
Carbaryl	10	0.000	Barley grain
		0.200	Potato
		10.000	Many fruits & veg.
Chlorpyriphos	1	0.500	Banana, plum
		0.100	Corn, fig, pumpkin
Diazinon	5	0.100	Banana, potato
		0.500	Many fruits & veg.
		0.750	Many fruits & veg.
Malathion	5	0.100	Flax seed
		0.200	Eggs
		1.000	Hop, sweet potato
		8.000	Many fruits & veg.
Parathion	5	0.010	Potable water (Clear Lake Region, CA)
		0.100	Nuts, potato, sugar beet
		1.000	Many fruits & veg.

a. As reported by MRI August, 1985.
b. Federal tolerances

performed in accordance with instructions provided by the manufacturer. Data was recorded at the time of testing and coded to enable comparison with results of chemical analysis.

Results and Discussion

The first objective of this study was to evaluate the practicality of using the tickets in the field to test well water samples. The personnel who collected the samples and used the tickets in the field found that, after resolution of a few sample handling problems, the tickets were practical for use in the field to test collected well water samples. The major problem encountered by the field personnel was keeping clean, pesticide-free glassware on hand in the field. This problem can be overcome by the use of disposable test vials.

The second objective of this study was to compare the results of conventional chemical analysis with results obtained from use of the tickets . Table IV shows the results of chemical and ticket analyses of the 11 wells. All tickets were negative for the presence of cholinesterase-inhibiting materials. Chemical analyses of six of the wells were positive for residues of one or more of the pesticides specifically analyzed for. None of the chemical screens of the wells were positive for organophosphate, chlorinated hydrocarbon or carbamate residues.

Since no cholinesterase-inhibiting pesticides were found, the EHAP was unable to completely evaluate the tickets. The negative results obtained from the tickets did match the negative chemical screening results for organophosphate and carbamate pesticides, but since no positive samples were found a complete evaluation could not be made. However, the following observations were made concerning the use of the tickets in EHAP's sampling program.

Table IV. Environmental Monitoring Study Results.

Chemical Analyzed For	Results of Conventional Chemical Analysis[a] (Range of 11 wells)	Results of EnzyTec® Pesticide Detector Ticket Analysis
Simazine	ND[b] - 0.81 (0.02)[c]	N.A.[d]
Atrazine	ND - 0.14 (0.02)	N.A.
Prometon	ND (0.02)	N.A.
Diuron	ND - 0.53 (0.05)	N.A.
Bromacil	ND - 0.13 (0.10)	N.A.
Organophosphate pesticide screen	ND (1.0)	ND (100)
Chlorinated hydrocarbon pesticide screen	ND (0.05)	N.A.
Carbamate pesticide screen	ND (1.0)	ND (100)

a. All values in parts per billion (ppb).
b. ND = None Detected (see c).
c. All values in parenthesis are minimum detectable amounts in parts per billion (ppb).
d. NA = Not Applicable.

1. The tickets are designed to detect only organophosphate and carbamate pesticides. The tickets do not detect other pesticides which may be found in California ground water, e.g., triazine herbicides. EHAP needs information on all pesticides which may be present.

2. The MDL of the tickets as currently formulated is too high. EHAP needs to know if pesticides are present in well water at any level. Table IV shows that conventional analytical methods have a much lower limit of detection than the tickets. This means that if the CDFA used the tickets to screen for organophosphate and carbamate pesticides, negative ticket results would still have to be tested by conventional chemical analysis to check for the lower levels that the tickets could not detect. Use of the tickets as a screening tool then would not save the CDFA any chemical analyses since both negative and positive ticket readings would have to be checked for lower level contaminate and specific compound identification, respectively.

IV. PESTICIDE USE ENFORCEMENT STUDY

The Pesticide Use Enforcement Branch of the CDFA collects agricultural commodity samples for pesticide residue analysis as well as performing required pesticide use enforcement investigations and duties. The Pesticide Use Enforcement Branch evaluated the tickets for use in screening of produce and other samples.

Objectives

The objectives of this study were to: 1) evaluate the practicality of using the tickets in the field to screen samples and, 2) to compare the results of conventional chemical analysis with results obtained from using the tickets.

Sampling Plan

The study used samples that were collected during routine Pesticide Use Enforcement work. Samples were collected in accordance with established EPA protocols. No attempt was made to select samples specifically for testing with the tickets. Samples were collected and tested both in the field and in CDFA laboratories in four areas of the state: Berkeley, Fresno, Anaheim, and Sacramento. Data were recorded at the time of testing and coded to enable comparison with results of chemical analysis.

The study was divided into two parts. In the first part, samples were collected and analyzed in the field using the tickets, then the remaining sample was transported to the laboratory for chemical analysis. Tickets were used on seven samples in Berkeley, five in Fresno, eight in Anaheim and eight in Sacramento. In the second part of the study, samples sent to the laboratory for chemical analysis were also analyzed in the laboratory using the tickets. In each laboratory, four commodity samples were each analyzed five times using the tickets.

All samples were generally screened in the laboratories for organophosphate, carbamate, and chlorinated hydrocarbon pesticides. Some samples were specifically analyzed for certain pesticides when it was known which pesticide residues might be present. All ticket testing was performed in accordance with instructions provided by the manufacturer.

Results and Discussion

The results of the field testing are presented in Table V. The results of the laboratory comparison study are presented in Table VI.

Table V. Results of field testing.

Sample Type	Result of Ticket	Result of Conventional Chemical Analysis	Comments
Berkeley			
Purple grapes	?[a]	NA[b]	Unable to read-pigmentation problems
Spinach	?	NA	Unable to read-pigmentation problems
Beets	?	NA	Unable to read-pigmentation problems
Mint	?	NA	Unable to read-pigmentation problems
Parsley	?	NA	Unable to read-pigmentation problems
Oranges (whole)	positive	negative	Sample too acidic
Parsley	negative	.08 ppm Dacthal®	Dacthal is a chlorinated hydrocarbon pesticide
Fresno			
Grape leaves (4 samples)	negative	NA	Known application of Naled (an organophosphate pesticide)
Clothing	negative	neg. Monitor® 0.37 ppm Curacron®	Organophosphate pesticide Organophosphate pesticide
Anaheim			
Grapes	negative	0.24 ppm Captan®	Organochloride pesticide
Plums	negative	0.06 ppm Botran®	Organochloride pesticide
Cucumbers	negative	0.14 ppm Thiodan®	Organochloride pesticide
Zucchini	negative	0.21 ppm Bravo®	Organochloride pesticide
Grapes	negative	0.67 ppm Demeton®	Organophosphate pesticide
Lima beans	negative	0.09 ppm Monitor® 0.98 ppm Acephate	Organophosphate pesticide Organophosphate pesticide
Lima beans	negative	0.11 ppm Monitor® 1.85 ppm Acephate	Organophosphate pesticide Organophosphate pesticide
Lima beans	negative	0.14 ppm Monitor® 1.13 ppm Acephate	Organophosphate pesticide Organophosphate pesticide
Sacramento			
Apple leaves (2 samples)	positive	NA	Known application of Imidan®

Table V
Page 2

Sample Type	Result of Ticket	Result of Conventional Chemical Analysis	Comments
Nectarine	positive	NA	Known applications of malathion, methoxychlor and captan
Apple leaves (2 samples)	negative	NA	Known application of Imidan®
Apple leaves	?	NA	Unable to read-pigmentation problems
Leaves	positive	30.8 ppm Guthion® 0.19 ppm Parathion 29.7 ppm Carbaryl	Organophosphate pesticide Organophosphate pesticide Carbamate pesticide
Shirt	positive	3.5 ppm Guthion 1.0 ppm Parathion 0.4 ppm Carbaryl	Organophosphate pesticide Organophosphate pesticide Carbamate pesticide

a. ? = Results unclear.
b. NA = Sample not analyzed.

Table VI. Results of laboratory comparison study.

Commodity Tested[a]	Results of Ticket Analysis	Results of Conventional Chemical Analysis[b]	Comments
Lemon	Positive	ND[c]	False positive due to acidic sample.
	Positive	ND	"
	Positive	ND	"
	Positive	ND	"
	Positive	ND	"
Grapefruit	Positive	ND	False positive due to acidic sample.
	Positive	ND	"
	Positive	ND	"
	Positive	ND	"
	Positive	ND	"
Grape	?[d]	ND	Color interference
	?	ND	"
	ND	ND	
	ND	ND	
	?	ND	Color interference
Grape	?	ND	Color interference
	?	ND	"
	?	ND	"
	?	ND	"
	?	ND	"
Tomato	ND	ND	
	?	ND	Color interference
	ND	ND	
	?	ND	Color interference
	ND	ND	
Watermelon	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
Watermelon	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	

Table VI
Page 2

Commodity Tested	Results of Ticket Analysis	Results of Conventional Chemical Analysis	Comments
Watermelon	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
Cantaloupe	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
Cantaloupe	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
Cantaloupe	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
Cantaloupe	ND	<0.1	Orthene® - organo-
	ND	<0.1	phosphate pesticide
	ND	<0.1	
	ND	<0.1	
	ND	<0.1	
Pears	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
Apples	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
Soil	?	ND	Color interference
	?	ND	"
	?	ND	"
	?	ND	"
	?	ND	"

Table VI
Page 3

Commodity Tested	Results of Ticket Analysis	Results of Conventional Chemical Analysis	Comments
Potato	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
Yam	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
Cherry	?	ND	Color interference
	?	ND	"
	?	ND	"
	?	ND	"
	?	ND	"
Lettuce	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
	ND	ND	
Spinach	?	ND	Color interference
	?	ND	"
	?	ND	"
	?	ND	"
	?	ND	"
Green onion	?	ND	Color interference
	?	ND	"
	?	ND	"
	?	ND	"
	?	ND	"

a. These samples were selected at random and the percent of samples having no detectable residues in or on them is consistent with our historical records.
b. All analyses in parts per million (ppm).
c. ND = None detected.
d. ? = Results unclear.

The first objective of this study was to evaluate the practicality of using the tickets in the field to screen commodity samples. Pesticide Use Enforcement staff encountered several problems with field use of the tickets. The first problem involves the manner in which the Federal and State laws require the sample to be prepared before analysis. In most cases the laws require that whole, unwashed commodity samples be liquified or blended in order to produce a homogeneous sample for analysis. The ticket directions also require the grinding/mashing of the sample before use (see Appendix I). The homogenation step is extremely difficult without an electric or mechanical food processor in the field. It is not practical from the standpoint of equipment/electrical needs or from the standpoint of added time in the field for inspectors to use the tickets at the collection site.

The second problem arises from the results of the ticket test conducted in the field. No matter what the results of the field tests the samples must still be sent to the laboratory for analysis as it must be determined if residues are present in excess of state and federal tolerance levels. In many cases, these tolerances are below the MDL of the tickets. A negative ticket result must be reanalyzed by conventional chemical analysis because levels present may be below the MDL of the ticket, and because non-cholinesterase-inhibiting pesticides may be present. A positive ticket test must be reanalyzed to determine the exact concentration of the pesticide or pesticides present. The added time needed to conduct the field ticket tests would not save the CDFA later testing in a laboratory but would add to the amount of work a field inspector must perform at each inspection site and therefore reduce the number of sites each inspector could visit daily.

The second objective of this study was to compare the results obtained using conventional chemical analysis with those obtained using the tickets. Four major areas of difference appeared here:

1. The tickets lack the sensitivity (lower detection limit) required by State and Federal laws for residue analysis. This often leads to false negatives.

2. The tickets give false positives due to the acidic nature of some commodities. For this reason the tickets could not be used on acidic foods such as citrus.

3. Commodities which contain strong pigmentation or which oxidize readily interfere with reading the tickets.

4. Both positive and negative ticket results must still be checked by conventional chemical analysis. Negatives may contain pesticides below the tickets high level of detection. Positives must be analyzed to determine the exact concentration of pesticide or pesticides present.

In summary, the CDFA would realize no budgetary or time savings if the Pesticide Use Enforcement Branch used the tickets. In fact, in some cases use of the tickets could increase time and expenditures.

V. WORKER HEALTH AND SAFETY STUDY

The Worker Health and Safety Branch in the CDFA conducts studies related to agricultural worker exposure or potential exposure to agricultural chemicals.

Objective

The objective of this study was to determine if the tickets could be used to determine worker re-entry times into a pear orchard which had been treated with azinphos-methyl.

Sampling Plan

A pear orchard in Sacramento County was monitored for the breakdown of azinphos-methyl residues. The product monitored was Gowan Azinphos-M 50WP® (EPA Reg. No. 10163-78 AA). Two applications were monitored. The application rates were 1 lb. active ingredient in 100 gallons of water per acre, and 1.5 lbs. active ingredient in 500 gallons of water per acre. Five samples were collected simultaneously at various time intervals after the applications. Each of the five samples consisted of 48 one-inch leaf disks, collected by using a Birkestrand leaf punch. Three samples were analyzed using a conventional gas chromatography method, and two samples were analyzed using the enzyme detector tickets provided by EnzyTec®, Inc.

Results and Discussion

All of the sample results reported from the gas chromatography analysis were above the minimum detectable limit of 0.005 ug/cm^2 . The lowest sample result was 0.15 ug/cm^2, reported at 39 days after the second application. Correspondingly, all of the sample results reported from the enzyme detector tickets were positive. Table VII gives the results of analysis of the collected samples.

Based on the results of this study, the tickets appear to adequately detect the presence of azinphos-methyl residues at levels above 0.15 ug/cm^2 . The estimated safe level for azinphos-methyl is 1.6 ug/cm^2. It appears that a negative enzyme

Table VII. Results of Worker Health and Safety Study.

Sample Date Collection	Days Post-Application	Azinphos-methyl Results in ug/cm^2*	x + S.D.		Enzyme Detector Ticket Results**
4/18/86	2	1.45, 1.32, 1.10	1.29	0.18	Both positive
4/30/86	14	0.47, 0.54, 0.46	0.49	0.04	Both positive
5/7/86	21	0.51, 0.51, 0.50	0.51	0.006	Both positive
5/13/86	27	0.35, 0.25, 0.38	0.33	0.07	Both positive
5/16/85	20	0.24, 0.28, 0.24	0.25	0.02	Both positive
Application rate was 1 lb. A.I. in 100 gallons of water per acre.					
5/17/86	3 hours	0.89, 0.65, 0.82	0.79	0.12	Both positive
5/17/86	7 hours	0.59, 1.12, 0.66	0.79	0.29	Both positive
5/18/86	1	0.77, 0.66, 1.03	0.82	0.19	Both positive
5/19/86	2	1.13, 1.05, 0.70	0.96	0.23	Both positive
5/20/86	3	0.61, 0.75, 1.03	0.80	0.21	Both positive
5/30/86	13	0.91, 0.60, 0.90	0.80	0.18	Both positive
6/5/86	19	0.31, 0.20, 0.27	0.26	0.06	Both positive
6/13/86	27	0.15, 0.21, 0.17	0.18	0.03	Both positive
6/17/86	31	0.19, 0.20, 0.21	0.20	0.01	Both positive
6/25/86	39	0.16, 0.16, 0.15	0.16	0.007	Both positive
Application rate was 1.5 lbs. A.I. in 500 gallons of water per acre.					

* MDL = 0.005 ug/cm^2 azinphos-methyl
** Two samples were taken, one enzyme ticket was run per sample.

ticket result is a reliable indicator of safe levels of azinphos-methyl residues on pear leaves. The manufacturer of the tickets has also supplied the CDFA with proposed procedures for determining worker reentry into fields sprayed with methomyl, methyl parathion or Diazinon®. The methods are presented in Appendices II, III and IV. The CDFA has made no attempt to experimentally validate these procedures.

VI. CONCLUSIONS AND RECOMMENDATIONS

The following conclusions and recommendations are based on the EnzyTec® Pesticide Detector ticket as currently formulated and apply only to the ticket's usefulness in the regulatory programs of CDFA.

1. The tickets work well for simple water sample screening. This is the procedure they were developed for. However, as presently formulated, the MDL of the tickets for pesticides is well above the MDL needed by CDFA's regulatory programs.

2. The tickets often produce an ambiguous color change at or near their limit of detection, even with standard solutions. This ambiguity can lead to different interpretations by different users. One user may judge a ticket as negative, while another may judge it as positive. This means that positive samples may be missed depending on the judgment of the user.

3. The tickets do not work well with highly colored commodities or products. The ether/hexane procedure suggested by MRI does show promise but will require a great deal of additional experimental work before it can be used routinely. The problem here is that this procedure is an extraction and once an extraction has been done, it is simple to analyze the sample by an existing instrumental procedure which will give more information than analysis by the ticket.

4. The tickets are not reliable for testing of fresh or canned commodities or products which are moderate to very acidic (pH less than about 3) or moderate

to very basic (pH greater than about 8). Some of these commodities and products are soft drinks, grapefruit juice, apple juice and cider, fruit jellies, lemons, limes, oranges, sour pickles, plums, brewed coffee and tea, strawberries, vinegar, and wines. Potable and non-potable water (e.g, irrigation or field tailwater) in some areas may also be too acidic or basic for use of the tickets.

5. The tickets give results which may be useful for checking re-entry intervals into sprayed fields. However, the method used for this type of testing requires the use of specialized equipment (a specific commercial branch of leaf punch) which may limit the method to use by only those with special equipment.

6. The tickets are not practical for field use. Commodities must be mechanically or electrically blended before testing. If the commodity or water sample is highly pigmented, the tickets cannot be used until the sample is extracted using procedures that are best performed only under controlled laboratory conditions. Since the tickets can give both false positives (e.g., sample is very acid) and false negatives (e.g., pesticide levels present below the MDL of the ticket) field readings must always be supplemented by conventional chemical analysis.

7. Because of all the limitations (pH, pigmentation etc.) the tickets should most likely be used only by trained personnel in situations where the user knows that a cholinesterase-inhibiting pesticide has been applied.

The EnzyTec® Pesticide Detector as currently formulated will not meet the requirements of the CDFA for use in its regulatory programs nor can CDFA

recommend their use by untrained personnel in the field. The tickets problems arise from the high detection levels of the tickets and their qualitative nature when not used in aqueous media. The California Department of Food and Agriculture recommends that further research and development work be done on the EnzyTec® Pesticide Detector to make the product more specific for cholinesterase-inhibiting pesticides and useable with media other than water.

APPENDICIES I-V

EnzyTec™

Pesticide Detection Systems

PRODUCT BULLETIN

PESTICIDE DETECTOR

U.S. Patent No. 4,324,858

STANDARD FEATURES

- Selective cholinesterase enzyme detector.
- Detects presence of organophosphate and carbamate pesticides.
- Simple Fail-Safe two-step ticket method with self-contained reagents.
- On-the-spot test produces results within minutes.
- Low cost detector with sensitivity in 0.1 to 10 ppm range.
- Easy to use without specialized training or knowledge.
- Long shelf life with no special storage requirements.
- Many applications including produce, fruit, water, air, industrial.

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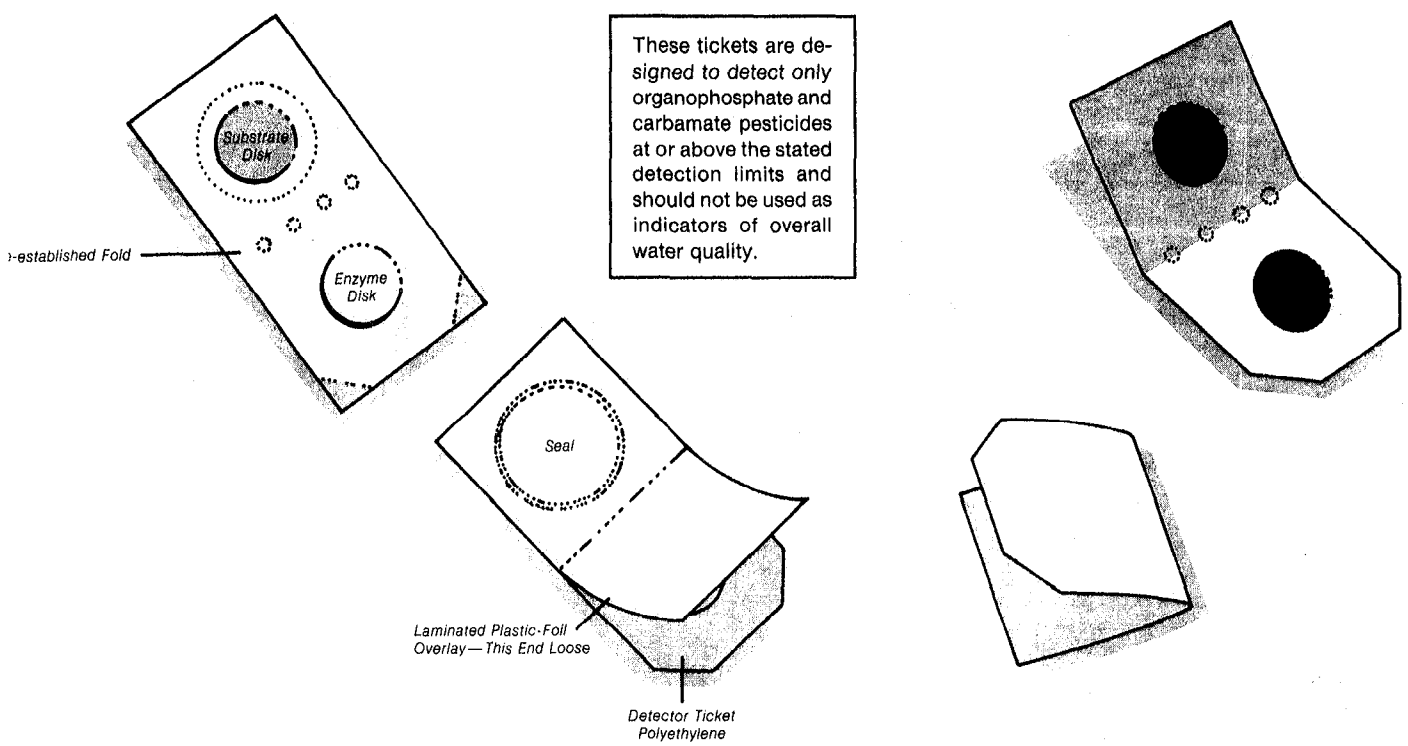
TYPICAL DETECTION LIMITS

COMMON NAME	CLASS	MINIMUM LIMITS ppm
Baygon	Carbamate	1
Furadan	Carbamate	1
Diazinon	Organophosphate	5*
Dursban	Organophosphate	1*
Malathion	Organophosphate	5*
Parathion	Carbamate	5
Sevin	Carbamate	10
Temik	Carbamate	1

* Treated with oxidizer to enhance sensitivity.

EASY TO USE



These tickets are designed to detect only organophosphate and carbamate pesticides at or above the stated detection limits and should not be used as indicators of overall water quality.

To perform a test, the enzyme disk (clipped corners end of the ticket) is moistened for 1 minute with a water sample taken from the material suspected of containing the pesticide. Next, the overlay is peeled off to expose the substrate disk. The ticket is then folded, bringing the two disks into contact for 3 minutes. The enzyme disk is examined for color.

A blue disk means that the sample being tested is safe. A white disk means that the sample may be contaminated.

The detector is FAIL-SAFE. A blue color can develop only if all components are functioning. A NO COLOR response will occur if any part of the ticket is inoperative or if a pesticide is present.

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ENZYTEC
PESTICIDE DETECTOR

The EnzyTec Pesticide Detector was developed by Midwest Research Institute (MRI), the fourth largest non-profit research institute in the United States, for detecting organophosphorus and carbamate pesticides in water. These two chemical classes of pesticides account for 85% of all insecticides sold yearly. The Pesticide Detector is one of a series of products developed by MRI for detecting pesticides in water and air.

The Pesticide Detector is sold in a simple ticket form and uses an enzyme, cholinesterase, to signal the presence of organophosphorus and carbamate pesticides. Because the detection method uses the same enzyme that is inhibited by pesticides in insects and mammals, the sensitivity of the ticket to various pesticides has a direct correlation with their toxicity. The Pesticide Detector has sensitivity in the 0.1 to 10 ppm range for most of the widely used organophosphates and carbamate pesticides.

The Pesticide Detector Ticket is "fail safe" in use and operates on a simple color-change principle. If the test sample is free of pesticide, the enzyme ticket reacts to produce a blue color. This color is produced only if all of the tickets components are functioning. Should any part of the ticket be damaged or inoperative, there will be no color change, warning the operator to check further.

The ticket is a quick, on-the-spot and inexpensive alternative to expensive laboratory analyses for a majority of applications. Sampling and test procedures for other applications are being developed. EnzyTec, Inc., will work with potential users to develop procedures to fit their individual needs. We trust that the additional information contained in this handout will be of use in determining whether this device can be used in place of the expensive procedure you are now using.

Table 1 contains information on the detection limit of many of the commonly used organophosphate and carbamate pesticides.

TABLE 1

TYPICAL DETECTION LIMITS

Pesticide		Detection Limit
Common Name	Other Name	(ppm, wt/vol)
Aldicarb	Temik	1
Propoxur	Baygon	1
Chlorpyrifos	Dursban	1*
Carbofuran	Furadan	1
Parathion	-	5*
Malathion	-	5*
Diazinon	-	5*
Carbaryl	Sevin	10

* Treated with bromine water to enhance sensitivity.

The Pesticide Detector was designed for detecting pesticides in water. Thousands of water samples have been tested and no materials in natural water, other than pesticides, have been found which inhibit the enzyme and thus interfere with the test. This enzyme, as are all enzymes, is a protein and can be degraded or destroyed by heat and a variety of chemicals when present in high concentrations. The detector will tolerate up to 10% methanol in the test media. Thus, pesticides can be extracted with this solvent if appropriate dilution with water is made before the test is conducted.

Many of the common organophosphate pesticides belong to a chemical class called thiophosphates. Thiophosphates are only weak cholinesterase inhibitors, but undergo metabolic or chemical oxidation converting them to their oxygen analogs which are stronger cholinesterse inhibitors. To increase the sensitivity of the ticket to such pesticides, the test sample is chemically oxidized with bromine water. A small sealed glass ampule of bromine water is included in the test kits with each ticket. It is necessary to use the bromine water to achieve the detection limits for thiophosphates noted in Table 1. Bromine water should be used for all samples suspected of containing thiophosphates or for samples for which the pesticide identity is unknown. The bromine oxidation step is not necessary when the pesticide being detected is known to be a carbamate or an organophosphate other than a thiophosphate.

A 1-minute contact time of the enzyme pad with the test media was used to achieve the detection limits shown in Table 1. A contact time of 10 minutes can increase the sensitivity of the ticket to pesticides by as much as a factor of 10. Contact times in excess of 10 minutes can result in resolution and loss of enzyme from the enzyme pad. Table 2 attached to this text identifies, by common name, most of the common thiophosphate, oxyphosphate, and carbamate pesticides sold commercially.

In addition to testing water, the ticket has been used to test for pesticides in air and on surfaces. The Pesticide Detector enzyme pad, exposed to air containing pesticides, will absorb the pesticide. When the enzyme pad is then wet with water, the pesticide is put into solution allowing it to inhibit the enzyme. Similarly, when the enzyme pad is contacted with a surface contaminated with pesticide and then wet or if the surface is swabbed or washed and the swab or wash solution used to wet the enzyme pad, the pesticides will be detected if present in sufficient concentrations.

The ticket can be used as a quantitative tool by taking advantage of the minimum detection limit and diluting your test sample until the pesticide can no longer be detected. This requires that the identity of the pesticide be known.

Many states, and in some cases, the USDA, have established limits for pesticide residues in foods and other products. Surface-absorbed pesticides can be detected by simply washing the produce; however, if necessary, the produce can be macerated in a blender and the resultant liquid media analyzed for pesticides. Again, using the minimum detection limit of the ticket, the sampling procedure can be structured to show whether the pesticide residue limit is exceeded. This same technique can be used to detect pesticide residue on leaf surfaces in orchards and groves where such residues pose a worker safety problem.

EnzyTec has available other pesticide detector systems based on this same detection principal. The detectors include continuous air and water monitoring instruments, and a portable quantitative air sampling device. All of these devices produce test results at a fraction of the cost of conventional laboratory analyses.

Inquiries regarding particular applications of the Pesticide Detector are most welcome.

TABLE 2

COMMON NAMES OF CHOLINESTERASE-INHIBITING COMMERCIAL PESTICIDES

Organophosphates		
Oxyphosphates	Thiophosphates	Carbamates
Acephate	Azinphos Methyl	Aldicarb
Chlorfenvinphos	Carbophenothion	Aminocarb
Crotoxyphos	Chlorpyrifos	Bendiocarb
Crufomate	Chlorthion	Carbaryl
Decamethyl triphosphoramide	Coumaphos	Carbofuran
Demeton	Demeton	Dimetilan
Demeton Methyl	Dialifor	Dixacarb
Dichlorvos	Diazinon	Formetanate
Dicrotophos	Dicapthon	Methicarb
Dimefox	Dimethoate	Methomyl
Fospirate	Dioxathion	Mexacarbate
Methamidophos	Disulfoton	Oxamyl
Mevinphos	EPN	Propoxur
Naled	Ethion	Pyrimicarb
Phosphamidon	Fenitrothion	
Prophos	Fenthion	
Schradan	Fonofos	
Stirophos	Malathion	
TEPP	Methyl Parathion	
Trichlorfon	Morphothion	
	Parathion	
	Phorate	
	Phosmet	
	Ronnel	
	Sulfotepp	
	Temephos	

INSTRUCTIONS

THESE TICKETS ARE DESIGNED TO DETECT ONLY ORGANOPHOSPHATE AND CARBAMATE PESTICIDES AT OR ABOVE THE STATED DETECTION LIMITS AND SHOULD NOT BE USED AS INDICATORS OF OVERALL WATER QUALITY

PLEASE READ CAREFULLY BEFORE STARTING TEST

1. These tickets expire January 1989. Discard any tickets that are outdated and reorder tickets.

2. Collect 20 mL of suspect water in the 50-mL beaker. Place the beaker on a hard, flat surface.

3. Remove packet of bromine tubes and open.

4. Place one bromine tube in the 20-mL water sample. The bromine should be a light brown color. If bromine is colorless, DO NOT USE.

5. Using the glass rod, crush the bromine tube against the bottom of the beaker, putting bromine into the solution. Stir until the brown color is no longer visible.

6. WAIT 3 MINUTES.

7. Open the enzyme ticket by tearing from notch to notch and remove its contents. Discard the white cardboard.

8. FOLD BACK (do not remove) the foil overlay exposing the enzyme disk. The "enzyme disk" is the disk located on the end which has the clipped corners. Dip the "enzyme disk" into the water for 1 minute.

9. Remove the ticket from the water and immediately peel off the folded back overlay, exposing the "substrate disk." After 15 seconds, fold the enzyme ticket in half so that the "enzyme disk" and the "substrate disk" will come in contact. With forefinger and thumb behind the disks, hold the disks together for 3 minutes.

10. After 3 minutes, open the ticket. Observe the color of the "enzyme disk" ONLY.

    BLUE COLOR - test is NEGATIVE. Any pesticide concentration is below the enzyme ticket's detection limits.

    WHITE COLOR - test is POSITIVE. Pesticides MAY BE present. Consider the test water "contaminated."

NOTE: A CONTROL TICKET may be run to verify the results. Rinse the beaker thoroughly with clean water and proceed with steps 8-10. (The enzyme ticket was designed to function in natural waters. High levels of chlorine in water may interfere with the test.)

BLUE COLOR - the enzyme tickets are performing correctly.

WHITE COLOR - the enzyme tickets are NOT performing.

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EnzyTec Data Sheet

Hexane Extraction From Aldicarb In Water

The Pesticide Detector Ticket will detect 1 ppm of aldicarb in water using a 1 minute incubation time. There are cases however, where the aqueous material being tested, contains chromaphores that mask the color produced by the Pesticide Detector Ticket.

The following procedure was developed to increase the sensitivity of the Pesticide Detector Ticket and to eliminate the problems encountered with aqueous chromaphores.

Procedure:

1. Prepare aqueous aldicarb standards using 0.5, 0.4, 0.3, 0.2 and 0.1 ppm. A water blank or control, no pesticide, should be run along with these standards as a reference.

2. Measure 20 mls of aldicarb solution and pour it into a 50 ml screw top glass test tube with a teflon lined cap.

3. Pipette 3 mls of hexane into the aldicarb solution. Screw the top on the test tube and invert slowly. The mixing of hexane and water produces a gas that must be carefully vented. Stop frequently and open the test tube to remove this gas pressure. When the water and hexane come to equilibrium, no pressure inside the test tube, then vigorously shake for 1 minute.

4. Most of the hexane will quickly rise to the top of the aqueous layer. However, some water droplets will remain in the hexane for a few minutes. Therefore, 5 - 10 minutes should be allowed for a good separation.

5. Remove the ticket from the kit. Tear open packet and remove silver-covered ticket.

6. Fold back loose end of silver cover to fold on ticket so that only white disc is exposed. END WITH CLIPPED CORNERS.

7. Using a glass disposable pipette, remove 0.5 mls of hexane. Add three drops of hexane to the enzyme disc, END WITH CLIPPED CORNERS, and allow the hexane to evaporate. Continue the addition and evaporation until all of the 0.5 mls of hexane has been added to the enzyme disc. Because there is ample hexane two or three tickets can be run for each extraction.

CAUTION

Be sure that the hexane is completely evaporated. Later in the test a color will be developed. If the hexane is not completely evaporated, blue spots will appear on the enzyme disc.

8. Add 3 drops of pesticide free water and wait 1 minute.

9. Remove the silver foil cover from the ticket, fold the ticket so that the two discs are together and hold between thumb and forefinger for 3 minutes.

10. Open ticket and immediately read the color on the enzyme disc, END WITH CLIPPED CORNERS. A white disc indicates that the aldicarb concentration is above the detection limit. A blue color indicates that the aldicarb concentration is below the detection limit. Aldicarb inhibited Pesticide Detector Tickets tend to give a blue tint upon standing. Therefore, it is necessary to read the results immediately.

11. It is suggested that the aldicarb extractions be done from highest to lowest. i.e., 0.5, 0.4, 0.3, 0.2, 0.1 ppm in order to determine the concentration where the blue color is formed.

The water blanks or controls, no pesticide, Step #1, will produce nice dark blue colors that can be used as a comparison.

NOTICE

While this procedure has been prepared to the best of our abilities, it is not to be construed as a warranty or representation or expected performance for which we assume any legal responsibility.

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WORKER REENTRY INTO DIAZINON TREATED FIELDS

The estimated safe level of foliar Diazinon residues for farm worker reentry into treated fields is 4.0 micrograms per square centimeter of leaf surface (1).

Following is a test procedure, using the EnzyTec Pesticide Detector Ticket, for determining whether the Diazinon residue is within the estimated safe level.

Water Test

Test the water to be used for the extraction to be sure it is free of detectable pesticides using the following procedure:

1. Pour 20 milliliters of water into a 50 milliliter beaker.
2. Add 1 bromine ampule to the 20 milliliters; crush with the glass rod, stir and allow to react for 3 minutes.
3. Place a Pesticide Detector Ticket in the 20 milliliters of brominated water for 1 minute. END WITH CLIPPED CORNERS INTO WATER.
4. Remove the Pesticide Detector Ticket, pull back the silver foil overlay, fold the two discs together and hold between thumb and forefinger for 3 minutes.
5. Separate the ticket and read the clipped end which should be a nice dark blue color. This indicates that there are no detectable pesticides in the water.

Test for Diazinon on Foliage

1. Collect forty (40) leaf samples using a leaf punch sampler, procedure and equipment described by Iwata et al. (2).
2. Place the leaf samples in a wide mouth 16 ounce bottle containing 405 milliliters of water and add 0.5 milliliter of a 1:50 dilution of Sur-Ten wetting agent.
3. Cap the bottle and shake vigorously for 1 minute.

4. Dilute a 10-milliliter aliquot of the extraction liquid to a final volume of 100 milliliters (mix well).

5. Place 20 milliliters of the diluted sample in the 50-milliliter test beaker.

6. Place 1 bromine ampule in the 20 milliliters water sample, crush the ampule with the glass rod, stir and allow to react for 3 minutes.

7. Immerse the Pesticide Detector Ticket in the brominated test sample for 1 minute. END WITH CLIPPED CORNERS INTO WATER.

8. Remove Pesticide Detector Ticket from the water. Pull back the silver foil overlay, fold the ticket so that the two discs meet and hold together for 3 minutes.

9. Separate the ticket and read the clipped end. Read the test results immediately. Note--Disregard any color change that may occur later. Ignore any individual small blue specks.

   A white disc indicates the dislodgable Diazinon residue on the leaf surface is above 4.0 micrograms per square centimeter. A blue disc indicates the concentration is below 4.0 micrograms per square centimeter.

   If one obtains a positive test, white disc, it is recommended that the test be repeated to confirm the results. This is a field screening test and the results obtained depend on many factors such as careful dilutions and the contact times of the Pesticide Detector Ticket to the test solution, etc.

Further information is available from our technical director, Mr. Bill Jacobs or Dr. Ivan C. Smith, President, EnzyTec, Inc., 425 Volker Boulevard, Kansas City, Missouri 64110 (816) 753-7600.

NOTICE

While this procedure has been prepared to the best of our ability, it is not to be construed as a warranty or representation or expected performance for which we assume any legal responsibility.

REFERENCES:

(1) Iwata, Y. R. C. Spear, J. B. Knaak, and R. J. Foster, "Worker Reentry into Pesticide-Treated Crops. I. Procedure for the Determination of Dislodgable Pesticide Residues on Foliage," Bull. Environ. Contam. Toxicol., 18, No. 6, 649-655 (1977).

(2) Maddy, K. T., "Estimated Safe Levels of Foliar Pesticide Residues to Allow Unprotected Workers Reentry into Treated Fields in California," Cal. Dept. of Food and Ag. Bull., HS-1280, Revised Dec. 16, 1985.

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WORKER REENTRY INTO METHYL PARATHION TREATED FIELDS

The estimated safe level of foliar Methyl Parathion on residues for farm worker reentry into treated fields is 0.6 micrograms per square centimeter of leaf surface (1).

Following is a test procedure, using the EnzyTec Pesticide Detector Ticket, for determining whether the Methyl Parathion residue is within the estimated safe level.

Water Test

Test the water to be used for the extraction to be sure it is free of detectable pesticides using the following procedure:

1. Pour 20 milliliters of water into a 50 milliliter beaker.

2. Add 1 bromine ampule to the 20 milliliters; crush with the glass rod, stir and allow to react for 3 minutes.

3. Place a Pesticide Detector Ticket in the 20 milliliters of brominated water for 1 minute. END WITH CLIPPED CORNERS INTO WATER.

4. Remove the Pesticide Detector Ticket, pull back the silver foil overlay, fold the two discs together and hold between thumb and forefinger for 3 minutes.

5. Separate the ticket and read the clipped end which should be a nice dark blue color. This indicates that there are no detectable pesticides in the water.

Test for Methyl Parathion on Foliage

1. Collect forty (40) leaf samples using a leaf punch sampler, procedure and equipment described by Iwata et al. (2).

2. Place the leaf samples in a wide mouth 8-ounce bottle containing 61 milliliters of water and add 5 drops of a 1:50 dilution of Sur-Ten wetting agent.

3. Cap the bottle and shake vigorously for 1 minute.

4. Place 20 milliliters of the diluted sample in the 50-milliliter test beaker.

5. Place 1 bromine ampule in the 20 milliliters water sample, crush the ampule with the glass rod, stir and allow to react for 3 minutes.

6. Immerse the Pesticide Detector Ticket in the brominated test sample for 1 minute. END WITH CLIPPED CORNERS INTO WATER.

7. Remove Pesticide Detector Ticket from the water. Pull back the silver foil overlay, fold the ticket so that the two discs meet and hold together for 3 minutes.

8. Separate the ticket and read the clipped end. Read the test results immediately. Note--Disregard any color change that may occur later. Ignore any individual small blue specks.

   A white disc indicates the dislodgable Methyl Parathion residue on the leaf surface is above 0.6 micrograms per square centimeter. A blue disc indicates the concentration is below 0.6 micrograms per square centimeter.

   If one obtains a positive test, white disc, it is recommended that the test be repeated to confirm the results. This is a field screening test and the results obtained depend on many factors such as careful dilutions and the contact times of the Pesticide Detector Ticket to the test solution, etc.

Further information is available from our technical director, Mr. Bill Jacobs or Dr. Ivan C. Smith, President, EnzyTec, Inc., 425 Volker Boulevard, Kansas City, Missouri 64110 (816) 753-7600.

NOTICE

While this procedure has been prepared to the best of our ability, it is not to be construed as a warranty or representation or expected performance for which we assume any legal responsibility.

REFERENCES:

(1) Iwata, Y. R. C. Spear, J. B. Knaak, and R. J. Foster, "Worker Reentry into Pesticide-Treated Crops. I. Procedure for the Determination of Dislodgable Pesticide Residues on Foliage," Bull. Environ. Contam. Toxicol., 18, No. 6, 649-655 (1977).

(2) Maddy, K. T., "Estimated Safe Levels of Foliar Pesticide Residues to Allow Unprotected Workers Reentry into Treated Fields in California," Cal. Dept. of Food and Ag. Bull., HS-1280, Revised Dec. 16, 1985.

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WORKER REENTRY INTO LANNATE (METHOMYL) TREATED FIELDS

The estimated safe level of foliar Lannate on residues for farm worker reentry into treated fields is 1.5 micrograms per square centimeter of leaf surface (1).

Following is a test procedure, using the EnzyTec Pesticide Detector Ticket, for determining whether the Lannate residue is within the estimated safe level.

Water Test

Test the water to be used for the extraction to be sure it is free of detectable pesticides using the following procedure:

1. Pour 20 milliliters of water into a 50 milliliter beaker.

2. Add 1 bromine ampule to the 20 milliliters; crush with the glass rod, stir and allow to react for 3 minutes.

3. Place a Pesticide Detector Ticket in the 20 milliliters of brominated water for 1 minute. END WITH CLIPPED CORNERS INTO WATER.

4. Remove the Pesticide Detector Ticket, pull back the silver foil overlay, fold the two discs together and hold between thumb and forefinger for 3 minutes.

5. Separate the ticket and read the clipped end which should be a nice dark blue color. This indicates that there are no detectable pesticides in the water.

Test for Lannate on Foliage

1. Collect forty (40) leaf samples using a leaf punch sampler, procedure and equipment described by Iwata et al. (2).

2. Place the leaf samples in a wide mouth 16-ounce bottle containing 608 milliliters of water and add 0.5 milliliters of a 1:50 dilution of Sur-Ten wetting agent.

3. Cap the bottle and shake vigorously for 1 minute.

4. Place 20 milliliters of the diluted sample in the 50-milliliter test beaker.

5. Place 1 bromine ampule in the 20 milliliters water sample, crush the ampule with the glass rod, stir and allow to react for 3 minutes.

6. Immerse the Pesticide Detector Ticket in the brominated test sample for 1 minute. END WITH CLIPPED CORNERS INTO WATER.

7. Remove Pesticide Detector Ticket from the water. Pull back the silver foil overlay, fold the ticket so that the two discs meet and hold together for 3 minutes.

8. Separate the ticket and read the clipped end. Read the test results immediately. Note--Disregard any color change that may occur later. Ignore any individual small blue specks.

   A white disc indicates the dislodgable Lannate residue on the leaf surface is above 1.5 micrograms per square centimeter. A blue disc indicates the concentration is below 1.5 micrograms per square centimeter.

   If one obtains a positive test, white disc, it is recommended that the test be repeated to confirm the results. This is a field screening test and the results obtained depend on many factors such as careful dilutions and the contact times of the Pesticide Detector Ticket to the test solution, etc.

Further information is available from our technical director, Mr. Bill Jacobs or Dr. Ivan C. Smith, President, EnzyTec, Inc., 425 Volker Boulevard, Kansas City, Missouri 64110 (816) 753-7600.

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